USDA United States Department of Agriculture

Animal and Plant Health Inspection Service

VS Veterinary Services

Operations & Necropsy Manual

SOSS

Scrapie: Ovine Slaughter Surveillance

Study 2002-2003

Table of Contents

This is a special edition of the 2002-2003 SOSS Manual. It does not contain either the cd-ROM nor the Laminated Visuals, both of which are nevertheless referred to throughout this manual. If you would like either of these items, please contact CEAH.

Introduction

Manual Objective

This manual is intended to replace the first SOSS Study Operations & Necropsy Manual assembled in April 2001. Changes to the manual include the addition of diagrams and pictures for use in the necropsy area. Additional anatomic diagrams are necessary due to the unique nature of many of the tissues. Also, it is imperative to minimize misidentification of tissues due to the costs of laboratory processing. The laminated color pictures are for individuals that are new to necropsy or that learn visually. The pictures are removable and can be taken to the slaughter plant or necropsy room.

This new manual will assist individuals directly involved with coordination and field operations. If the need arises, further updates will be made. You'll also find a SOSS Study cd-ROM kiosk in the back of this binder.

USDA

United States Department of Agriculture

Marketing and Regulatory Programs

Animal and Plant Health Inspection Service

Washington, DC 20250

SUBJECT: Implementation of SOSS To Help Eradicate Scrapie JAN 2 2002

TO: Regional Directors
Area Veterinarians in Charge
Veterinary Medical Officers

Scrapie is estimated to cost U.S. producers $20-25 million annually. Infected flocks are less productive, as affected animals typically die during their peak productive years. The disease is often spread before the animals appear to be infected. Recent attention to TSEs and to the possible link between BSE and the feeding of cattle in England with scrapie-infected sheep products has severely affected domestic and international trade in sheep and sheep-derived products. Many renderers have declined to render sheep offal or to pick up dead sheep, significantly increasing disposal costs. In addition, other countries have indicated possible restrictions on importing certain non-sheep ruminant products from the United States because of scrapie.

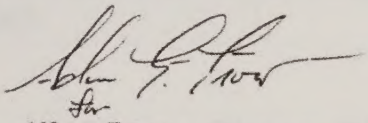
APHIS has made a commitment to the sheep industry to eradicate scrapie by 2010. To accomplish this eradication, we are implementing the SOSS program, which stands for Scrapie: Ovine Slaughter Surveillance. We will first use the SOSS program to estimate the prevalence of scrapie in U.S. mature cull ewes that enter slaughter channels and to determine the best sheep population to target for sampling in the future and the resources needed for eradication. SOSS will then be used as the basis for establishing an effective slaughter surveillance mechanism to identify infected flocks for regulatory action, since rapid identification of scrapie-infected flocks is critical to the eradication effort.

CEAH has produced a manual to provide guidance to VS Area offices and field employees participating in the SOSS program. The manual focuses on field operations and necropsy, and is written to be understood by the entry level animal health technician. The manual will be made available to all VS participants in the SOSS program.

APHIS - Protecting American Agriculture

We consider implementation of the SOSS program critical to our efforts to eradicate scrapie from the United States.

Sincerely,



Alfonso Torres
Deputy Administrator
Veterinary Services

Roles and Responsibilities

Veterinary Services and State Animal Health Officials

Employees will be involved in the proper selection and sampling of ovine heads. They also will determine the state of origin of each sampled animal. *It is very important that the Sample Submission/Traceback form is accurate and complete, as this information will be used in the analysis.* A coordinator will handle local field logistics in each state conducting slaughter sampling. Traceback efforts will also be handled through the state conducting the sampling. Other states may be involved in this effort as well.

National Animal Health Programs

The National Animal Health Programs (NAHPS) is headquartered in Riverdale, Maryland. NAHPS is responsible for the direction, facilitation, policy, and budget for the scrapie program and the SOSS study.

Centers for Epidemiology and Animal Health, Center for Animal Health Monitoring

Located in Fort Collins, Colorado, the Centers for Epidemiology and Animal Health (CEAH) provides technical expertise for the design and planning of the SOSS Study 2002-2003.

National Veterinary Services Laboratory

The National Veterinary Services Laboratory (NVSL) is located in Ames, Iowa. NVSL is coordinating the laboratory support for the SOSS Study 2002-2003. There are three laboratories contracted to assist NVSL with laboratory testing: Colorado State University, School of Veterinary Medicine, Veterinary Diagnostic Laboratory; University of Wyoming, State Diagnostic Laboratory; and Qiagen Inc., Bothell, Washington.

Food Safety Inspection Service

The USDA Food Safety Inspection Service (FSIS) is responsible for inspecting slaughter plant facilities and the United States meat supply. FSIS has agreed to assist Veterinary Services (VS), when needed, in gaining cooperation from plant owners. VS field employees are responsible for initiating plant contact and providing information about the study.

Table 1 lists the mature-sheep slaughter plants selected to participate in the SOSS Study 2002-2003:

Table 1. Mature-Sheep Slaughter Plants Participating in SOSS

Blue Mountain
Monticello, Utah

Ruwaldt Packing
Hobart, Indiana

Strube Packing
Ballinger, Texas

Berry & Sons
Detroit, Michigan

Ranchers Lamb
San Angelo, Texas

Riverside Livestock
Farmington, New Mexico

Wolverine Packing
Detroit, Michigan

Rhode Island Beef & Veal
Johnstown, Rhode Island

Hilltown Pork
Canaan, New York

Halal Meat
Paterson, New Jersey

Halal Fresh
Newark, New Jersey

Louie Chiu
Washington, New Jersey

Lemay & Sons
Goffstown, New Hampshire

Halal Packing
Newton, New Jersey

Hampton Meat
Hopkinsville, Kentucky

Harmon Brothers
Walton, Kentucky

Henry Kohn
Monroeville, New Jersey

Stagno's Meat
Modesto, California

Stephen Manteri
Bechtelsville, PA

Noor Halal
Imler, Pennsylvania

C & L Ranch
Fruitland, New Mexico

Laird Sheep
Benton, Kentucky

Safety and Health Requirements

The National Institute of Occupational Safety and Health Services (NIOSH), as well as the Animal Plant Health Inspection Service (APHIS) Employee Health Services, have reviewed current scientific literature and occupational safety and health standards regarding the SOSS Study.

By studying this manual, VS employees performing tissue collection should be familiar with proper animal selection, tissue collection, and submission of samples. Each selected state has a SOSS Study State Coordinator. These coordinators are responsible primarily for oversight and implementation of the study in their respective states, including reporting all injuries and illnesses of suspected occupational origin.

APHIS personnel and Federal Occupational Health centers are available for assistance. For a location near you, go to **http://www.foh.dhhs.gov**.

Whether tissue collection is on-site (slaughter plant) or off-site, choose an area with adequate space to allow for safety.

A. The following are work practice requirements for VS employees:

1) Do not eat, drink, or smoke in work areas where animal or animal tissues/fluids are present.

2) When contact may occur with live animals, tissues, or fluids, such as during actual collection of sheep heads or during the necropsy procedure, **protective clothing and equipment must be worn (as discussed on page 7.).**

3) Place plastic-backed absorbent pads or a sheet on the table during necropsy to minimize aerosol (or mist). Sample the ovine head while it is in the plastic bag, to facilitate clean up.

4) Contact the area office or area veterinarian for current rabies pre-exposure prophylaxis, tuberculin skin test, tetanus, and diphtheria immunization.

5) Seek immediate first aid for cuts or other injuries. Report all injuries to the SOSS Study state coordinator and the Federal Occupational Health office in your area. Coordinators will submit injury reports to the area office.

6) Use protective clothing and equipment. Protective clothing should not be worn in employees' vehicles or while eating. Remove protective clothing before leaving the site and, when appropriate, dispose of properly. Disinfect clothing using color-safe or regular bleach solution.

7) Wash hands and exposed skin before and after tissue sampling, and whenever gloves are removed.

8) Use 50:50 sodium hypochlorite (bleach solution) to disinfect surfaces, equipment, and instruments.

9) Dispose of sharp objects in a sharps container.

B. Personal protective equipment (PPE) is required for VS employees involved in tissue collection for the SOSS Study:

1) Wear coveralls or a lab coat to protect street clothes. Whenever possible, use disposable garments.

2) Wear eye protection (safety glasses or goggles).

3) Use a face shield. This is a secondary protector designed to provide limited protection to the face and front of the neck.

4) Wear disposable gloves. Heavy rubber gloves should be available for cleaning and disinfecting surfaces.

5) If on-site (slaughter plant), wear a hard hat, rubber boots, and earplugs. A disinfecting footbath should be available before and after entry to the site.

C. To obtain information about additional PPE not required, consult Mr. Peter Petch at the APHIS: Safety & Health office: 301.734.5383.

D. Individuals who are immunocompromised should contact their personal physician for advice regarding their participation in the SOSS study.

Sheep Selection

How to select sheep to sample

For Phase II, VS field employees will use **systematic sampling**. Sheep are selected based on the number of animals needed for a given day (**See Table 2**). After the first animal is randomly selected, proceed by selecting every **n**th individual, where **n** depends on the number of samples available at the slaughter plant at the time. For example, you would collect 15 heads if 150 animals are presented in a stratum 2 plant), then select every 10th head (150/15 = 10). In stratum 3, 8 heads are needed each week. If 50 sheep are slaughtered on the collection day, then select every 6^{th} head (50/8 = 6.25).

If the desired number of sheep is not present on the day of collecting, take what is there. For stratum 4 plants, there is some flexibility. This may mean getting fewer than 3 heads, if 3 sheep are not available one week and more are available the next week (**do not collect more than 6 heads in one week**). If there will be a stretch of time when no ewes will be slaughtered at the plant (seasonal variation) give CEAH a call. We can work out a strategy to make up the numbers without overwhelming the lab.

Identification and Traceback

This section addresses how state-of-origin determinations should be made for the SOSS study. The SOSS study state coordinators are responsible for reporting traceback information to CEAH within 30 days of sampling. After each sampling session, the traceback form must be completed and sent to the state coordinator. It is very important that these forms are as complete and accurate as possible.

All sheep over 18 months old should have official identification tags. However, we know that some sheep entering slaughter channels do not have proper/official identification. In general, there are three situations we expect you to encounter: 1) sheep with no identification; 2) sheep with limited identification that arrive in identifiable lots; 3) sheep with proper/official identification. Each of these situations is discussed below and shown in a completed sample traceback form on page 11.

1) Sheep with no identification: Sheep in this group have no traceable identification and cannot be attributed to a particular lot. However, do not make this determination until the heads have been thoroughly examined for ID (eartags or tattoos) and records are checked to see if the sheep are part of a lot that can be identified to a particular sale, buyer, or state. Any sheep that meet the "no identification" criteria need to be recorded as "UNK". These animals will still be sampled, but the results will only be used to calculate estimates for the U.S. prevalence of scrapie, not regional estimates.

Example 1: You sample a head with no discernible identification. The animal is a black face with incisors I1 and I2 fully in wear and I3 just beginning to erupt. You check with your plant contact to see if an origin can be determined. Unfortunately, the answer is "no"... The sheep arrived in 1 of 2 lots, one of which originated in over 20 states. It took you 10 minutes to try tracing this animal. Record that time on the traceback form.

2) Sheep with limited identification: These are sheep that lack individual traceable identification but can still be identified to a particular lot or group. In these cases, it is important to determine the origin of the group and record it by listing all of the states that particular lot of sheep came from.

Example 2: You sample a head bearing a red 10 tag. You check with the plant before leaving for the day and find that the group of sheep the animal came from was delivered by a buyer from a neighboring state who frequents several sales. In checking with the Animal Identification Coordinator (AIC) from that state, you discover that this buyer attends sales that

include sheep from over 10 states. In this case, you record the origin state as "UNK". Your attempts to trace this white-faced animal took 30 minutes.

Example 3: You sample a black-faced head with a broken mouth and a bright orange tag numbered 134. You check with the plant before leaving and find that this animal was consigned by the owner, a Mr. X from a neighboring town in Colorado. You check with Mr. X to verify the state of origin. In this case you record the origin state as Colorado. It took you 15 minutes to determine this animal's state of origin.

Example 4: You sample a 4-year-old, speckled-face head with a green 101 tag. You check with the plant before leaving and note that the consignor was Mr. J. Trader. Mr. Trader is known to you/your AIC as a local buyer who picks up culls from Colorado, Nebraska, and Wyoming. You record these possibilities in the traceback form's state of origin field. It took 10 minutes to gather this information.

Example 5: You sample a skinned head with I3 incisors and a yellow tag numbered 39. Your plant contacts tell you that this animal came through the XYZ Market. You know that nothing can be traced through the XYZ Market without an official ID. Therefore, you record the state of origin as "UNK".

Example 6: You sample a head with a red 14 tag. The plant tells you this animal came from the ABC Market just down the road. You call them and explain that you want to find the animal's state of origin. You are told that this animal was consigned by a local farmer who lives in Colorado. It took 10 minutes to complete the traceback for this animal.

Sheep with official identification: When a sheep arrives with official identification, record all the information on the tag so that the study coordinator can call/email the state where the tag was issued and find out where the animal originated. Since tags may have been issued to dealers, markets, and individuals that do not represent a farm of origin, it is best that these determinations be made by someone in the state responsible for issuing the tags. General Data Base (GDB) macros have been written to assist in determining the appropriate state of origin designation.

Example 7: You sample a white-faced head with the following tag: WY20850148. You contact the Designated Scrapie Epidemiologist (DSE) in Wyoming who refers you to the Wyoming AIC. The AIC runs the SOSS tag macro and gets the following output:

LOW TAG	HIGH TAG	ST	OPER TYPE	DIST. DATE
WY20850101	WY20850200	WY	DEALER	31-JAN-02

The AIC contacts the dealer the tag was issued to and finds that the animal came from either Wyoming or Nebraska. In all, your time and the time of those in Wyoming totaled 30 minutes. Record that on the traceback form.

Scrapie: Ovine Slaughter Surveillance
TRACE BACK - PHASE II

Collection Date: 4/16/02 Primary Collector: Judy R Plant Name: New Bedford - 9999

Number of Samples Collected Today: 7 Total number of MATURE sheep slaughtered at the plant today: 48

	SOSS Sample # on Label	Official ID	List Other ID's	Face Color (circle one)	Age by teeth (circle one)	State Last Resided for Breeding	How was trace back done? (check all that apply) (e.g., used GDB, plant, market, &/or dealer records)	Minutes to trace back this tag
Example								
1	10014	none		White (Black) Mottled Unknown	1yr (2yr) 3yr 4yr broken	unk	☐ GDB ☐ Tag ☑ Plant ☐ Market ☐ Dealer ☐ other-specify:	10
2	10015		Red1ϕ	(White) Black Mottled Unknown	1yr 2yr (3yr) 4yr broken	unk	☐ GDB ☐ Tag ☑ Plant ☑ Market ☐ Dealer ☐ other-specify:	30
3	10016		OR134	White (Black) Mottled Unknown	1yr 2yr 3yr 4yr (broken)	CO	☐ GDB ☐ Tag ☐ Plant ☐ Market ☐ Dealer ☑ other-specify: owner	15
4	10017		GR1ϕ1	White Black (Mottled) Unknown	1yr 2yr 3yr (4yr) broken	CO NE WY	☐ GDB ☐ Tag ☑ Plant ☐ Market ☐ Dealer ☐ other-specify:	10
5	10018		YEL39	White Black Mottled (Unknown)	1yr 2yr (3yr) 4yr broken	unk	☐ GDB ☐ Tag ☑ Plant ☐ Market ☐ Dealer ☐ other-specify:	3
6	10019		Red14	White (Black) Mottled Unknown	1yr (2yr) 3yr 4yr broken	CO	☐ GDB ☐ Tag ☑ Plant ☑ Market ☐ Dealer ☐ other-specify:	10
7	10020	WY2085ϕ148		(White) Black Mottled Unknown	1yr 2yr (3yr) 4yr broken	WY NE	☑ GDB ☐ Tag ☐ Plant ☐ Market ☐ Dealer ☐ other-specify:	30
				White Black Mottled Unknown	1yr 2yr 3yr 4yr broken		☐ GDB ☐ Tag ☐ Plant ☐ Market ☐ Dealer ☐ other-specify:	
				White Black Mottled Unknown	1yr 2yr 3yr 4yr broken		☐ GDB ☐ Tag ☐ Plant ☐ Market ☐ Dealer ☐ other-specify:	
				White Black Mottled Unknown	1yr 2yr 3yr 4yr broken		☐ GDB ☐ Tag ☐ Plant ☐ Market ☐ Dealer ☐ other-specify:	

How many hours were spent at the plant today? 1.5 hours

How many hours were spent driving to and from the plant today? .75 hours

When trace back is completed, send this yellow copy to CEAH.

g/cahm/sheep/soss/submission form.lwp

Table 2. Sampling allocation by plant using FSIS Fiscal Year 2001 data

Stratum	FSIS Plant #	State	FSIS 2001 head	No. Sites	FSIS (total head)	n
1	market	TX	174,000	1	174,000	7,500 for 50 wk period **(150 per week)**
2	05502	IN	22,784			3,750 for 50 wk period **(15 per week)**
	02825	UT	22,770			
	10252	MI	15,532			
	02574	MI	13,067			
	13029	TX	13,034			
				5	87,187	
3						
	07183A	NM	8,268			1,600 for 50 wk period **(8 per week)**
	02875	CA	6,061			
	08083	KY	5,461			
	17778	NJ	5,310			
				4	25,100	
4						
	07019	NM	3,004			1,950 for 50 wk period **(3 per week)**
	04018	NY	2,856			
	09381	PA	1,871			
	20403	NJ	1,781			
	08850	NJ	1,733			
	08616	PA	1,633			
	07429	KY	1,621			
	05910	NJ	1,538			
	05911	NJ	1,408			
	05300	RI	1,061			
	09542	NH	1,351			
	07356	KY	1,037			
	*19651	TX	1,000			
				13	21,894	
Total			**237,418 (fy01)**	23	**308,181**	**14,800**
22 plant total			**134,181**			

***Note: This plant reported by FSIS at 9014 for fiscal year 2001. Mature slaughtered is approximately 1000.**

151,434 FSIS total adjusted mature slaughter for four plants and estimated 368,000 to Mexico.

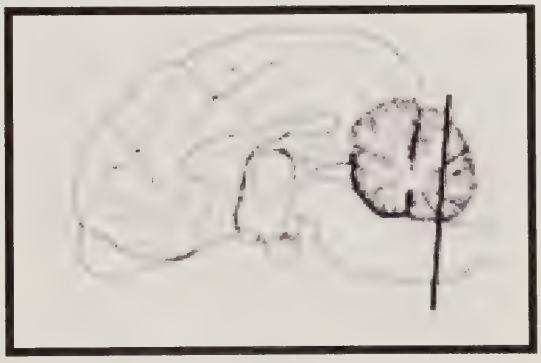
Sheep Anatomy

Obex

The obex is situated at the caudal end (V-shaped) of the fourth ventricle of the brain. Dorsal to the opening of the central canal of the medulla ventricles are internal brain cavities, which are continuous with the central nervous system of the spinal cord. The fourth ventricle is situated between the cerebellum and the medulla oblongata. The obex region of the brain, specifically the motor nucleus of the vagus, is a critical area to collect for scrapie diagnosis (See Figures 2 to 5 on the following pages).

Modified from *Miller's Anatomy of the Dog*, 2nd edition; Evans, Howard; Christensen, George; W.B. Saunders Company; ppg. 843

Figure 1. Dorsal view of obex



Modified from *Functional Mammalian Neuroanatomy*, 2nd edition; Jenkins, Thomas; Lea & Febiger; ppg. 28

Sheep Anatomy

The obex is a primary tissue for scrapie disease diagnosis. It will be used along with the tonsil to determine a scrapie-disease positive animal. The obex is found in brain tissue. Review the following figure and digital images before attempting sample collection.

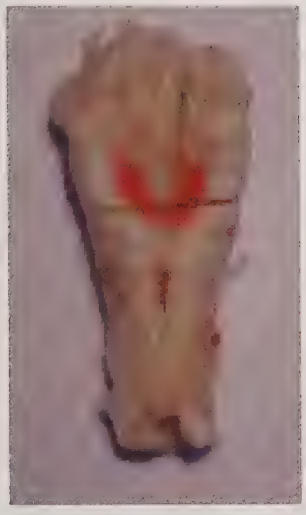
In the following figures, compare the stained and unstained motor nucleus of the vagus. For tissue collection, the goal is to cut the brain stem into 2 pieces (cranial and caudal portions) such that each half contains a portion of the motor nucleus of the vagus.

Courtesy National Veterinary Services Laboratories

Figure 2. Brain stem with obex: The section on the left contains a portion of the cerebellum. The motor nucleus of the vagus is dyed orange on the section at the right. Notice the location of the cut area on the section at the right. This is the cut to be used for obex tissue collection.

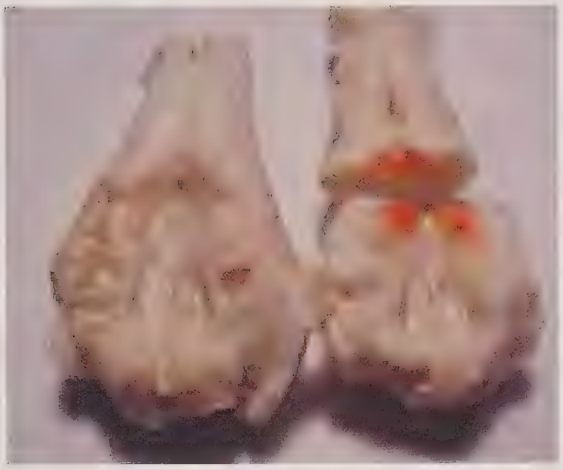
Sheep Anatomy

Obex Digital Images



Courtesy National Veterinary Services Laboratories

Figure 3. Obex: The motor nucleus is dyed red. The position of the preferred cut is shown.



Courtesy National Veterinary Services Laboratories

Figure 4. Two sections of obex: Compare the red-dyed motor nucleus on the right with the non-dyed motor nucleus on the left.

Figure 5. Section of obex: The motor nucleus is dyed red.
The position of the preferred cut is shown.
The cranial portion (top) is placed in formalin.
The caudal portion (bottom) containing the spinal cord is placed in its own self-locking bag and shipped on ice.

Courtesy National Veterinary Services Laboratories

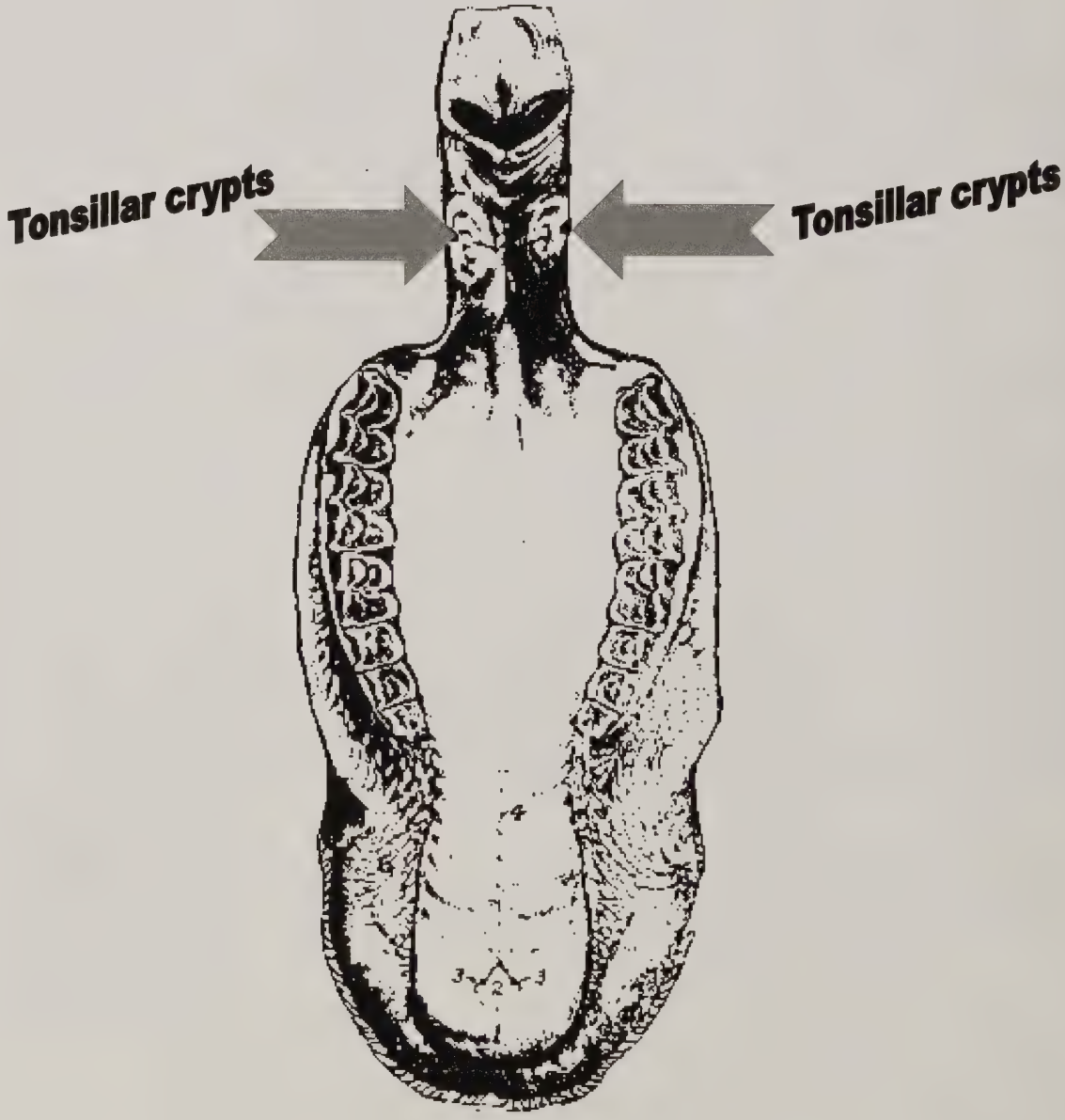
Sheep Anatomy

Tonsils

In sheep, tonsils are aggregations of lymphatic tissue in the soft palate. On the lateral walls near the attachment of the soft palate are the openings of the tonsillar crypts, which lead into the palatine tonsil. The palatine tonsil in sheep is about 12 mm long (See Figures 7 & 8).

For tissue collection, both tonsils are submitted for study.

Figure 6. Sheep palate (ventral view)



Anatomy of Domestic Animals 6th edition,
Pasquini et al, Sudz Publishing ppg. 459

Sheep Anatomy

Tonsils

See tonsil diagram (figure 6 on previous page); close-up pictures (figures 7 and 8 on following page); and tonsil video clip in the cd-ROM Kiosk (inserted at the back of manual).

The tonsil should be removed after the obex and cerebellum are collected.

Tools necessary for tonsil removal are: scalpel; sharp/sharp scissors; and rat-toothed forceps. Position your body so that the jaw of the mandible is facing upward, and the nose of the sheep is facing you.

Upon set-up of the head, skin (when present) and subcutaneous tissues are cut on the midline, and reflected to the side (lateral). This allows for easy visibility of the jaws of the mandible.

To remove the tongue, utilize the inner (medial) aspect of both jaws as landmarks to make a deep 'V" incision. Cut underlying connective tissues at the mandibular symphysis, and along the inside of the mandible.

Reflect the tongue away from you (caudally) as far as tissues allow. Observe the soft palate. At this point, you should be able to observe the tonsillar crypts on both sides (bilaterally). The tonsillar crypts lead to the tonsil. Make a V-shaped incision into the roof of the soft palate, rostral to the tonsillar crypts.

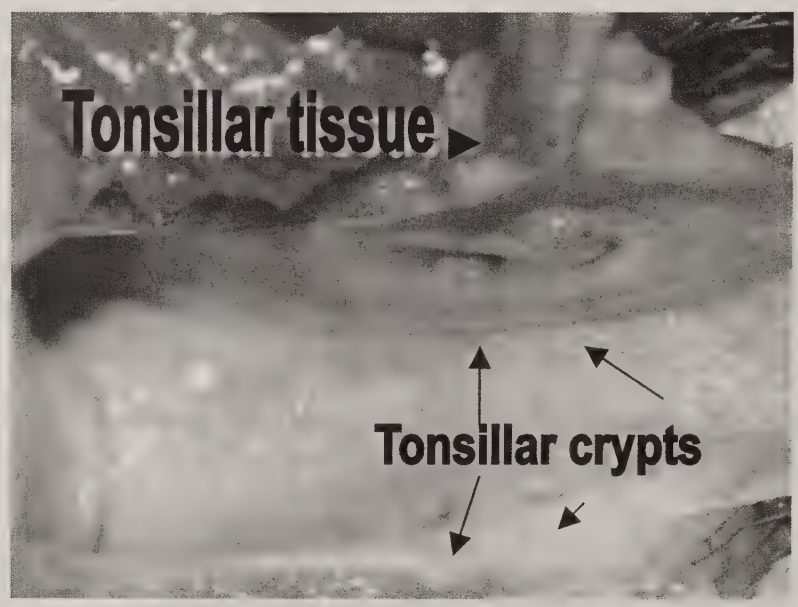
Observe the hyoid bones on both sides (bilaterally). There are two sites of joints (articulations). The tonsillar crypts are located on the inner aspect (medial) to the hyoid apparatus. Disarticulate the hyoid bones.

After the hyoid bones are disarticulated, cut down the inner (medial) aspect of the hyoid bone closest to you (proximal). Continue to sever all muscles and connecting tissues above (dorsal) the soft palate while pulling the tongue caudally. At this point, you may opt to completely remove the tongue.

Locate the tonsillar crypts in the lateral wall of the soft palate. Upon observing the crypts, follow them to the tonsil. They are easily observed by passing the forceps into one of the crypts. The tonsil is an aggregate of glandular tissue with a light yellow or white (fat-like) appearance, which ranges from 5 to 12mm in length, depending on age.

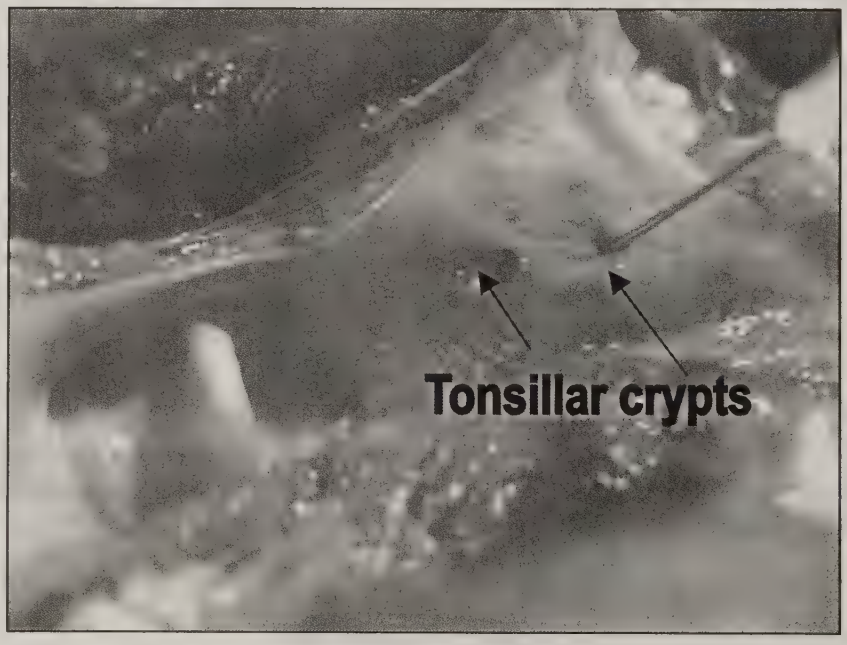
Sheep Anatomy

Tonsil Digital Images



USDA/FSIS Training Center

Figure 7. Close-up view of tonsillar crypts that lead to tonsillar tissue.



USDA/FSIS Training Center

Figure 8. Close-up of tonsillar crypts, identified by arrows and needle.

Sheep Anatomy

Retropharyngeal lymph nodes

The medial and lateral retropharyngeal lymph nodes are paired tissues located in the head. Lateral retropharyngeal lymph nodes are relatively superficial compared to medial retropharyngeal lymph nodes. For the SOSS Study, two lymph nodes, either medial or lateral retropharyngeal, are collected.

Lateral retropharyngeal lymph nodes are situated ventromedial to the wing of the atlas, located caudoventral to the origin of the digastricus muscle and dorsal to the carotid artery. These lymph nodes lie on the vagus, sympathetic, and accessory nerves. They are embedded in fatty connective tissue and covered by the caudodorsal portion of the mandibular gland. The lymph nodes vary in size from 4.0 to 5.0 cm long and 2.0 to 3.5 cm wide. Often, 1 larger lymph node is accompanied by 1 to 3 smaller lymph nodes. Medial retropharyngeal lymph nodes are situated medial to the stylohyoid bone, embedded in fatty connective tissue on the dorsolateral face of the pharyngeal muscles, between the larynx and atlas. Usually, there is one lymph node, 3.0 to 6.0 cm long and 2.5 to 4.0 cm wide. This is the largest lymph node of the head and neck.

Figure 9. Ventral view of medial and lateral retropharyngeal lymph nodes With esophagus and trachea reflected rostrally.

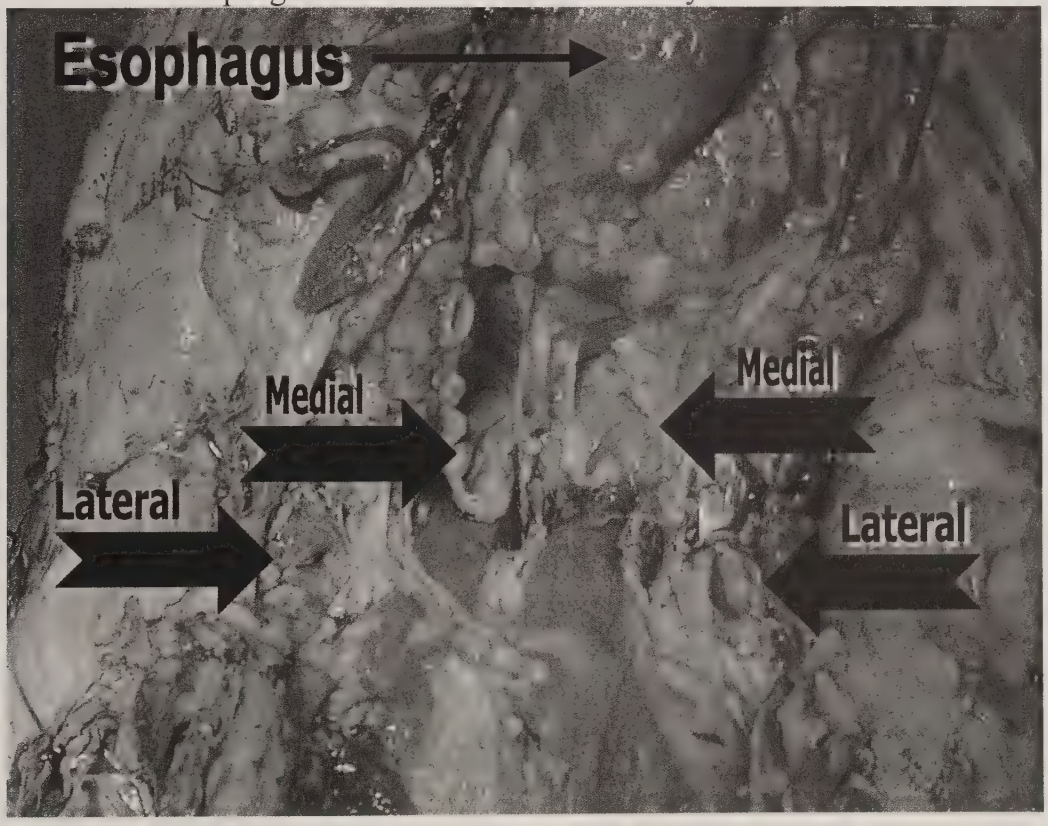


Photo by Denise Hall, DVM

Necropsy Procedures

Tissue Collection Summary

Note: Make sure all tissues from the same animal are labeled with the same SOSS sample number.

Prior to tissue collection, review safety and health recommendations for the SOSS Study.

The following tissues are needed:

- Obex (**See Figures 1 to 5, starting on page 13**).
- Both tonsils (**See Figure 6 on page 16, and Figures 7 and 8 on page 18**).
- Two retropharyngeal lymph nodes (**See Figure 9 on page 19**).

Water Extraction Technique

Review the SOSS Study cd-ROM Kiosk for this procedure.

Figure 10. Picture of water gun used for water extraction technique.

The water extraction technique uses a water gun (See Figure 10). Attach the gun to a suitable water source. Turn the water source on. Insert the copper tubing below (dorsal) the visible brain stem. Squeeze the handle and the tissues should 'pop-out' into your hand.

Necropsy Procedures

Brain Scoop Technique

Review the SOSS Study cd-ROM Kiosk for this procedure.

Using forceps and scalpel, trim away connective tissues surrounding the visible portion of the brain stem. Apply the brain scoop tool alongside the brain, following the opening toward the nose (rostrally). Rotate the tool clockwise and counterclockwise. This should sever the brain stem and cerebellum from the rest of the tissues. Pull the brain scoop tool back (caudally), and the brain stem and cerebellum should 'pop-out.'

European Brain Scoop Technique

See SOSS Study cd-ROM Kiosk for this procedure.

Cerebellum

Remove the cerebellum and place a large portion in an empty jar and label with SOSS sample number. Do not cross contaminate between sheep. Clean and wipe tools (with bleach solution) after each completed tissue collection from each head. Blood from one animal may interfere with DNA from another animal.

Each specimen jar and bag must be labeled with the sample number. Printed labels are provided in each kit (See page 25).

Do NOT include information for official sheep identification, flock/premise, or slaughter plant on the specimen jars and bags!

Necropsy Procedures

Obex

Review figures 3 to 6. At this point, the cerebellum should be removed. Locate the brainstem with the distinctive "V". This is the obex. Cut the brainstem into two pieces (cranial and caudal portions). The cut should be made approximately 1 mm cranial to the point of the "V".

Identify the "fleshy pink" motor nucleus of the vagus. In the figures of the obex, the section containing the motor nucleus is dyed red. The goal is to cut the brain stem into two pieces such that each half contains a portion of the motor nucleus of the vagus.

Place the cranial portion of the obex in the formalin jar and label the jar with the SOSS sample number.

Do NOT include information for official sheep identification, flock/premise, or slaughter plant on the specimen jars and bags!

Tonsils

Collect both tonsils. When possible, trim away excess tissue. Leave the overlying epithelium in place. Put one tonsil in the formalin jar. Place the other tonsil in a separate self-locking bag, label both the jar and bag with the SOSS sample number.

Retropharyngeal lymph nodes

Collect 2 retropharyngeal lymph nodes (either medial or lateral). Place one lymph node in the formalin jar. Place the other lymph node in a separate self-locking bag, and label the jar and the bag with the SOSS sample number.

Quality Control

Make sure that specimen jars and bags are properly labeled (i.e.: same SOSS number, use for all tissues from same head)
Thoroughly review paperwork for completeness.
Clean and wipe tools between each head with bleach solution.
When collection is finished, disinfect tools in bleach solution for one hour.
Inspect tools for future use. Return unusable tools to NVSL along with specimens.
Dispose of scalpels in sharps container.
ONLY use these tools for SOSS Study.
Properly dispose of plastic bags, paper, and debris in a safe manner.
Wash and clean clothing/equipment after each day's collection.

Necropsy Procedures

Specimen Collection Materials for Phase II

Collection materials are available for the SOSS Study. SOSS Study state coordinators are responsible for monitoring the supply of items that are bulk-ordered.

Prior to Phase II, the coordinator in each selected state will distribute bulk-ordered items to the prosectors. Bulk items include:

- Formalin jars.
- Empty jars for cerebellum
- 2 x 2 Ziploc bags.
- 4 x 4 Ziploc bags.
- Razors and disposable scalpels.
- Ice packs.
- Submission forms.
- Labels.
- Airbills.
- Shipping boxes.

Separate shipping boxes are used. One box is for formalin-fixed tissues, the other for fresh tissues. Fresh tissues are to be kept on ice packs(provided in the box).

Specific tools (listed below) for Phase II are provided by NVSL. The prosectors are to evaluate tools at the end of each collection. Return worn-out tools to NVSL. Contact the coordinator in your state for replacements. NVSL will send two sets of tools for each prosector.

- The following tools are needed for each collection:
- 2 pair of rat-tooth forceps.
- 4 pair of curved-curved scissors.
- 2 brain extraction scoops or one water extraction tool.

When sampling, the tools should be wiped off and disinfected (use tray with 50:50 hypochlorite solution) between ovine heads to prevent cross-contamination.

These tools are to be thoroughly cleaned and disinfected at the end of the collection day and reused for the next collection period.

Necropsy Procedures

Phase II Kits

Unlike Phase I, Phase II will not have set kits. Bulk materials will be sent to field staff specified by the coordinator. The field will reorder supplies as materials run low.

Initial shipment will include enough fomalin jars for approximately 6 months. Additional materials, such as the scalpels, razors, bags, and empty jars will be sent in bulk and replenished as needed.

Items provided by Area Office (monitored by SOSS Study State Coordinator):

1) Personal protective equipment.
2) Sharpie pens
3) Rubber and/or latex gloves.
4) Tape for formalin lids (black electrical).
5) Sodium hypochlorite (bleach).
6) Sharps container.
7) Clear plastic bags for heads if needed.

Remember!!!

The tonsil, lymph nodes, and caudal portion of the brain stem MUST be in separate bags.

Shipping Procedures

Summary of Tissue Collection

In Formalin

- Obex (cranial portion of brain stem).
- 1 tonsil.
- 1 retropharyngeal lymph node.

Send samples without ice.

On ice or frozen

- Cerebellum (placed in empty jar).
- 1 tonsil (placed in separate, 2 x 2 self-locking bag).
- 1 retropharyngeal lymph node (placed in separate, 2 x 2 self-locking bag).
- Obex (caudal portion of brain stem with attached spinal cord. Placed in separate 2 x 2 self-locking bag).
- Label the three bags and place them into a 4 x 4 bag.

Send samples with ice packs.

Specimen Labels

Phase II specimen kits will contain pre-printed labels with unique sample numbers.

A set of labels will look something like this:

PHASE II#10001 Formalin Obex, Tonsil, Retro Lymph node	**PHASE II#10001** Empty Jar Cerebellum	
PHASE II#10001 Self-lock Bag Tonsil	**PHASE II#10001** Self-lock Bag Caudal portion w/cord	**PHASE II#10001** Self-lock Bag Retro Lymph node

Shipping Procedures

Shipping

Complete the form in its entirety. Send the pink and gold colored pages to NVSL with the samples.
Retain the yellow copy for traceback data. Make a copy of the original for your office records. Send the original form to:

Ms. Judy Rodriguez, USDA- APHIS: Veterinary Services
555 South Howes
Fort Collins, Colorado 80521
970.494.7842

After May 2002, CEAH's new address will be:

USDA:APHIS:VS:CEAH
National Animal Health Monitoring System
2150 Centre Avenue, Bldg. B
Fort Collins, Colorado 80526
970.494.7000

Samples are to be shipped for overnight delivery. Avoid weekend and holiday delivery.

Samples MUST arrive in good condition. For chilled samples, use plenty of ice packs to ensure samples stay cold. If regular ice is used, use another bag (double bagging) to prevent leakage, and use plenty of absorbent materials.

If not shipped the same day as collection, freeze the tissue samples. If possible, ship with dry ice, or use plenty of ice packs. Keep the samples frozen.

For the formalin samples, make sure that lids are placed squarely on the jar and hand tightened. Tape shut. Using a sharpie, write the sample number on the top of the jar. Place jars inside the large plastic bag and close with twist-tie to contain any leaks. Place in a shipping container (separate from the bagged tissues). **Do not** send formalin samples on ice.

Use the addressed Federal Express airbills enclosed with the Phase II sampling boxes.

ALL SAMPLES go to:

Mr. John Ely, USDA: NVSL
1800 Dayton Avenue
Ames, Iowa 50010
515.663.7212

Forms Completion

Tissue Collection Form

Refer to page 11 to see Tissue Collection Form.

1st Column	SOSS# for each ovine head (**write in**).
2nd Column	Official sheep identification (**write in**).
3rd Column	List other animal identification (**write in**).
4th Column	Face color (**circle**).
5th Column	Age (**circle**).
6th Column	State of Origin (**write in**).

Phenotype (Face Color)

Review laminated insert.

Circle the face color.

- **White**, **Black**, **Mottled** (both colors present), **Unknown.**

Age

Review Appendix 3. Roll down the lower lip to examine incisors.

Classification	
1 year	The central incisors erupt at 1 to1.5 years.
2 years	The I2 teeth erupt at 2 to 2.5 years.
3 years	The I3 teeth erupt at 3 to3.5 years.
4 years	The I4 teeth erupt at 4 to 4.5 years.
5 years or older	Broken mouth. These animals may have broken teeth.

State of Origin

Enter the state of origin: state where the animal was born; or the state it last resided for breeding purposes. Do traceback using the yellow copy. If there is no official sheep identification and the animal is not traceable, write in 'Unk' for unknown.

Scrapie

Fact Sheet: Veterinary Services August 2001

Scrapie is a fatal, degenerative disease affecting the central nervous system of sheep and goats. It is among a number of diseases classified as transmissible spongiform encephalopathies (TSE). Infected flocks that contain a high percentage of susceptible animals can experience significant production losses. Over a period of several years the number of infected animals increases, and the age at onset of clinical signs decreases making these flocks economically unviable. Animals sold from infected flocks spread scrapie to other flocks. The presence of scrapie in the United States also prevents the export of breeding stock, semen, and embryos to many other countries. TSEs are the subject of increased attention and concern because of the discovery of bovine spongiform encephalopathy (BSE) in cattle, the link between BSE and variant Creutzfeldt-Jakob disease (vCJD) in people, and feline spongiform encephalopathy (FSE) in cats in Europe. This increased concern has led to the following:

- Packers and producers have had difficulty finding options for disposal of sheep offal and dead sheep causing packers and producers to incur significant increases in disposal costs,
- Other countries have expressed concerns and have indicated that they may prohibit or restrict certain ruminant products because the United States has scrapie, and
- Domestic and international markets for U.S. sheep-derived meat and bone meal have adversely affected.

The combination of all of these factors has led to the decision to develop a strong scrapie eradication program in the United States.

Epidemiology and Transmission

The agent responsible for scrapie and other TSEs is smaller than the smallest known virus and has not been completely characterized. There are three main theories on the nature of the scrapie agent: (1) the agent is a virus with unusual characteristics, (2) the agent is a prion, which is a malformed protein in the brain, and (3) the agent is a virino, a very small piece of DNA that acts like a virus. The scrapie agent is extremely resistant to heat and to normal sterilization processes. It does not evoke any detectable immune response or inflammatory reaction in host animals.

The scrapie agent is thought to be spread most commonly from the ewe to her offspring and to other lambs through contact with the placenta and placental fluids. Signs or effects

of the disease usually appear 2 to 5 years after the animal is infected but may not appear until much later. Sheep may live 1 to 6 months or longer after the onset of clinical signs, but death is inevitable. The genetics of the sheep affects their susceptibility to scrapie.

In the laboratory, the scrapie agent has been transmitted to hamsters, mice, rats, voles, gerbils, mink, cattle, and some species of monkeys by inoculation. There is no scientific evidence to indicate that scrapie poses a risk to human health. There is no epidemiologic evidence that scrapie of sheep and goats is transmitted to humans, such as through contact on the farm, at slaughter plants, or butcher shops.

Clinical Signs

Signs of scrapie vary widely among individual animals and develop very slowly. Due to damage to nerve cells, affected animals usually show behavioral changes, tremor (especially of head and neck pruritus, and locomotor in coordination that progresses to recumbency and death.

Early signs include subtle changes in behavior or temperament. These changes may be followed by scratching and rubbing against fixed objects, apparently to relieve itching. Other signs are loss of coordination, weight loss despite retention of appetite, biting of feet and limbs, lip smacking, and gait abnormalities, including high-stepping of the forelegs, hopping like a rabbit, and swaying of the back end. An infected animal may appear normal if left undisturbed at rest. However, when stimulated by a sudden noise, excessive movement, or the stress of handling, the animal may tremble or fall down in a convulsive-like state.

Several other problems can cause clinical signs similar to scrapie in sheep, including the diseases ovine progressive pneumonia, listeriosis, and rabies; the presence of external parasites (lice and mites); pregnancy toxemia; and toxins.

On the farm, veterinarians diagnose scrapie based on the appearance of its signs combined with knowledge of the animal's history. There is a test currently undergoing evaluation by the U.S. Department of Agriculture's (USDA) Animal and Plant Health Inspection Service (APHIS) for scrapie detection in live animals. Scrapie can currently only be confirmed by microscopic examinations of brain tissue at necropsy or by procedures that detect the presence of the abnormal prion protein.

Research

Scrapie research efforts are currently focused on developing a practical live-animal test to diagnose infected sheep before they show signs, investigating transmissibility of the agent, identifying the scrapie agent and its different strains, identifying genes that influence scrapie infection and evaluating genetic selection as a tool for scrapie control, and examining the role of artificial insemination and embryo transfer in the transmissibility of the scrapie agent. Research studies using

experimentally infected sheep suggest that embryos may play a role in the spread of scrapie.

Related Diseases

The TSE family of diseases includes BSE: transmissible mink encephalopathy; FSE; chronic wasting disease of deer and elk; kuru; both classical and variant Creutzfeldt-Jakob disease; Gerstmann-Straussler-Scheinker syndrome; and fatal familial insomnia. TSEs have also been reported in Europe in captive wild ruminants in the bovid family, cats, and monkeys. The occurrence of TSEs in captive wild animals is believed to have resulted from BSE-contaminated feed.

Control Program

USDA has initiated an accelerated scrapie eradication program. The program is based on the following key concepts:

- Identification of preclinical infected sheep through live animal testing and active surveillance.

- Effective tracing of infected animals to their flock/herd of origin made possible as a result of the new identification requirements, and

- Providing effective cleanup strategies that will allow producers to stay in business, preserve breeding stock, and remain economically viable.

APHIS will do this by providing the following to exposed and infected flocks/herds that participate in cleanup plans:

1. Indemnity for high-risk, suspect, and scrapie positive sheep and goats, which owners agree to destroy,

2. Scrapie live-animal testing

3. Genetic testing, and

4. Testing of exposed animals that have been sold out of infected and source flocks/herds.

Operating an effective program to deal with this insidious disease requires cooperation among producer organizations, allied industries, and governmental agencies.

History

First recognized as a disease of sheep in Great Britain and other countries of Western Europe more than 250 years ago, scrapie has been reported throughout the world. Only two countries are recognized by the United States as being free of scrapie: Australia and New Zealand.

The first case of scrapie in the United States was diagnosed in 1947 in a Michigan flock. The flock owner had imported sheep of British origin through Canada for several years. From this first case through July 2001, scrapie has been

diagnosed in more than 1,000 flocks in this country.

In the United States, scrapie has primarily been reported in the Suffolk breed. It also has been diagnosed in a Border Leicester, Cheviots, Corriedales, a Cotswold, Dorsets, Finn sheep, Hampshires, Merinos, Montadales, Rambouillets, Shropshires, Southdowns, and a number of crossbreeds. Through August 2001, approximately 1,600 cases in sheep and 7 cases in goats have been reported.

Additional For more information:
www.aphis.usda.gov/vs/scrapie.htm

Appendix 1. Contact Information

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Appendix 2: References

1. *Functional Mammalian Neuroanatomy*, Second Edition; Jenkins, Thomas; Lea & Febiger; pgs. 25, 26, 35, 200

2. *The Anatomy of the Domestic Animals Volume One*, Getty, Robert. W.B. Saunders Company; ppgs. 865,1025

3. *The Anatomy of the Domestic Animals Volume Two*, Getty, Robert, W.B. Saunders Company, Chapter 57

Appendix 3: Sheep Aging

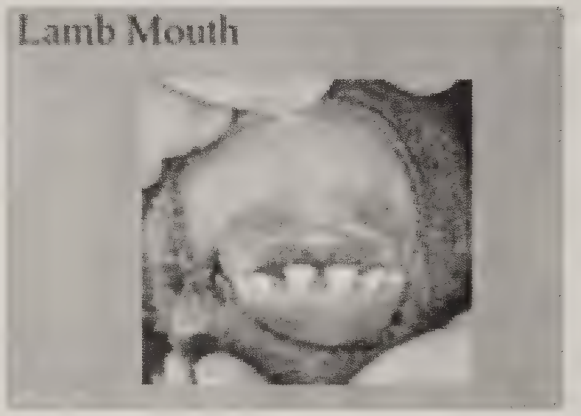

Figure 11. Observe the size of the first incisors (I1)

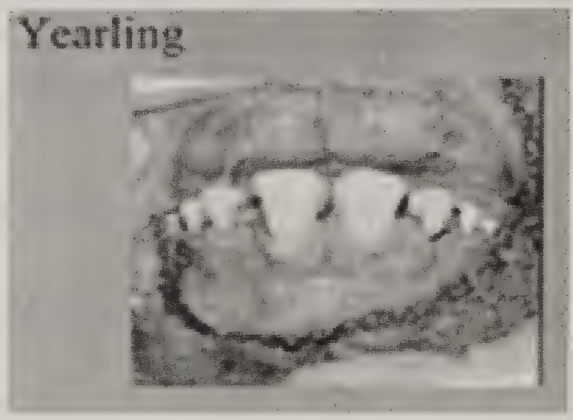

Figure 12. Observe the size of the first incisors (I1)

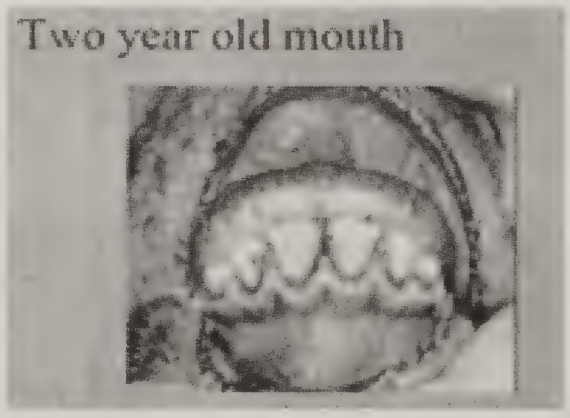

Figure 13 . Observe the first incisors (I1), and the incisors (I3) eruption of the second incisors (I2).

Figure 14. Observe the eruption of the third

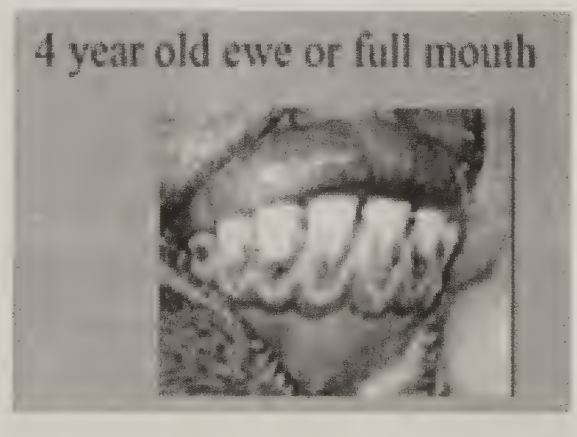

Figure 15. Adult sheep with a full mouth (all years. Incisors erupted).

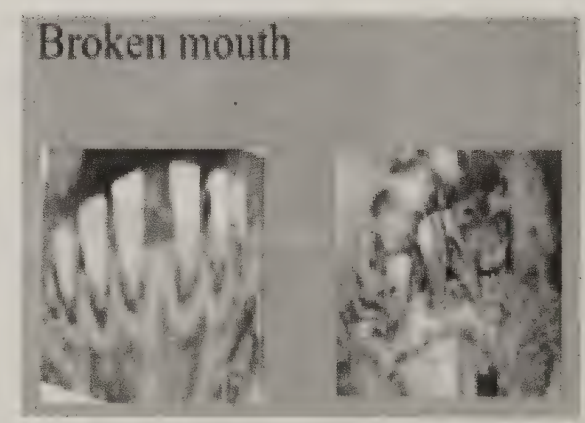

Figure 16. Broken mouth in sheep older than 5

Photos by Eileen Kuhlman

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